STUDY ON EFFECT OF SELECTED ORGANOPHOSHATE INSECTICIDE CHLORPYRIFOS ON BIOCHEMICAL, HAEMATOLOGICAL AND HISTOPATHALOGICAL ALTERATION IN FRESH WATER FISH *LABIO ROHITA* (HAMILTON, 1822)

A

Synopsis

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INTRODUCTION

The Green revolution was a period that began in the 1960s during which agriculture in India was converted into a modern industrial system by the adoption of technology, such as the use of high yielding variety seeds, mechanical farm tools, irrigation facilities, pesticides, and fertilizers. India is a global agricultural powerhouse. It is the second largest producer of rice, wheat cotton, fruits, farmed fish, vegetables, and tea. After green revolution during sixties especially in India, pesticides emerged as knight armors for crops (Shrivastva and Singh 2014).

In general, pesticides are used very extensively in agriculture, forestry, public health and veterinary practices and gaining immense importance due to their ability to control weeds, pests including insects, plant diseases, aquatic weeds, and aquatic snails (Gagnair et al, 2004;Jain et al,2005; Mustapha, 2008; Naeem et al,2010;Abu –Darwish et al,2011). Pesticides are categorized into 3 major types according to their target used are insecticides, fungicides, herbicides. The major chemical groups of pesticides used are organophosphate, carbamate, organochlorine, pyrethroid, trizole, necotenoides (Shrivastava and Singh, 2014; Sarba and Mehana, 2015).

Water is most precious natural resource on the earth. Plants, animals, and human beings, all need water to survive. Aquatic toxicology is study of the effects of environmental pollutants on aquatic organism. Agricultural runoff near water is the major cause of deposition of pesticides in aquatic ecosystem.

For increasing agricultural production and productivity use of chemical inputs such as pesticides has increased. Fishes serve as important bioindicators for aquatic contamination. Since recent studies indicated that fishes are quickly becoming scarce owing the increasing use of chemical pesticides in fields. Since fishes are important sources of proteins and lipids, heath of fishes is very important for human beings (Srivastava and Singh, 2013a).

In Nanded district mainly grown crop is cotton, banana, soybean, sugarcane and turmeric. To avoid the attack of pests on these crops, the farmers are using various types of pesticides. The excess use of such pesticides directly or indirectly causes harmful effect on aquatic life. Some study shas been done on effect of pesticides on freshwater fishes but very few studies have been conducted on the insecticide chlorpyrifos. **Chlorpyrifos** (**CPS**), also known as Chlorpyrifos ethyl, is an organophosphate pesticide that has been used on animals, and buildings, and in other settings, to kill several pests, including insects and worms on various crops particularly on cotton plants (Chakra Reddyand and Venkateswara, 2008). Its half-life in soil is about one week (Tomlin, 1994). It acts on the nervous systems of insects by inhibiting the acetylcholinesterase enzyme (Thomas C et al, 2020). One reason for the extensive use of PFF is because of its short half-life in soil, however, it has been recognized as highly persistent and toxic pesticide even at low concentrations (Zhao et al., 2008). Chlorpyrifos is considered moderately hazardous to humans (Class II) by the World Health Organization based on acute toxicity information dating to 1999. High exposure cause neurological effects, developmental

disorders, and autoimmune disorders. Exposure during pregnancy may harm the mental development of children (Israel, Brett 2012).To find out the harmful effects of these chemical substances as well as find out some alternative ways to control the pests, it is very necessary to carry works on diversity of organisms by applying various concentrations of pesticides.

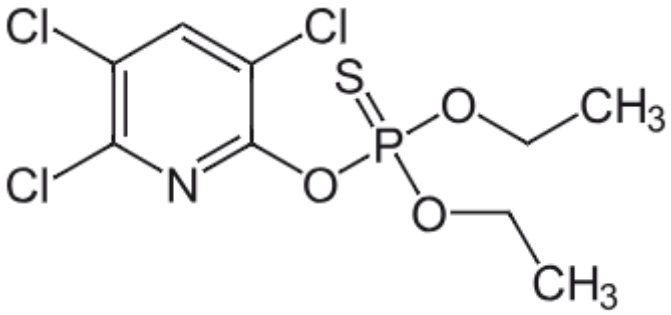

Fig: Chemical structure of chlorpyrifos

In study region the availability of literature on the toxicity of chlorpyrifos in several species of fresh water fish, a detailed analysis of the toxicity, biochemical and histological levels in the gill (site of exposure), liver (site of metabolism) and kidney (site of excretion) is lacking. Therefore, the present investigation is aimed at elucidating chlorpyrifos-induced toxicity in the edible major carp *Labeo rohita,* commonly known as Rohu. *Labeo rohita* is a member of the Indian major carp present in river and pond. Being one of the prime aquaculture species of the world, it occupies a prominent position in an aquatic ecosystem. Godavari is one of the greatest river in india containing variety of aquatic life specifically different species of teleosts. Extensive use of pesticides leading to build-up of pesticide residues in such edible fish population poses a potential threat to human health. Therefore, data on toxic effects of pesticides on *Labeo rohita* can help in toxicological risk assessment caused by pesticides to human beings and to the ecosystem as a whole.

CLASSIFICATION OF LABEO ROHITA : ROHU

Phylum:- CHORDATA (Notochord and dorsal tubular nerve cord present and gill-slits present).
Group:- CRANIATA (Cranium with brain present).
Subphylum:- VERTEBRATA (Vertebral column present).
Divisio :- GNATHOSTOMATA (Jaws and paired appendages present).
Super class :- PISCES (Paired fins, gills and skin with scales)
Class :- OSTEICHTHYES (Bony fishes).
SUB-CLASS :- ACTINOPTYERGII (Ray finned fish).
SUPER ORDER :- TELEOISTEI (Bony fish proper).
Order :- CYPRINIFORMES (Anterior vertebrae fused. Webrian ossicles present between air bladder and ear).
GENUS :- *Labeo*
SPECIES :- *rohita*

REVIEW OF LITERATURE

1) Pallavi Srivastava, Ajay Singh and A. K. Pandey1(2016) reviewed, the pesticides toxicity in fishes biochemical, physiological and genotoxic aspects and concluded that pesticides of almost all the groups do have acute toxic effects of various fish species.

2) Khanapure and Mudkhede (2013) investigated the Rasbora daniconius is more susceptible to organophosphate dimethoate than the other fish species. The dimethoate effects on different factors of water content like temperature, pH, D.O etc. As temperature increased, it reduces the solubility of oxygen in water which cause lethal effects on fishes.

3) Ghanbahadur A. and Ghanbahadur G. (2012) reviewed the histopathological effect of organochloride endosulphan on intestine and stomach of carnivorous fish Rasbora daniconius. The test fish exposed to for acute and chronic concentration. The fish showed severe damage when exposed to chronic than acute one.

4) Ghanbahadur A. *et al*., (2012) investigated, effect of pH on endosulphan toxicity in Rasbora daniconius. The fish exposed to insecticide endosulphan showed behavioral changes such as excitation, excess mucus secretion on body surface, fast movement, equilibrium loss and try to escape from waterbody and cause death. The above observation indicates that in Rasbora daniconius themortalitypercentage increased with increase in pH concentration.

5) Nikam S.M, et.al., (2011) They have studied the acute toxicity of organophosphate pesticide. Metasystox have been studied on fresh water fish *Nemachellus botia*. Fish became restless and started moving to & flow in water.Fish started coming on the surface of water to inhale air thus started suffocating within 24 hrs. Paralysis of body parts was observed in fishes. The LC50 valvesand exposure period showed a direct relationship.

6) T. SHIVALINGU Dr. U.M. JAYBHAYE(2021) They have studied effect of selected organochlorine pesticides on biochemical, reproductive, haematological and respiratory aspects of *CYPRINUS CARPIO* , Dieldrin and Methoxychlor was taken as lethal concentration and it shows the biochemical (protein, carbohydrates and lipids in gills, liver and brain) responses of the fish, haematological alterations, reproductive alterations and respiratory alterations.

OBJECTIVES OF THE RESEARCH

- To study the test indicator freshwater fish *Labio rohita* with its detailed characters and classification.
- Chemical parameter analysis of water sample (a medium in which the subject of this investigation inhabits).
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- The study of toxicity evaluation (determination of LC50) of chlorpyrifos insecticide on *Labio rohita.*

- Study of respiratory metabolism with total Oxygen consumption and rate of Oxygen consumption under the influence of chlorpyrifos insecticide and compared the control set.

- To study the effect of insecticide on hematology including RBC, WBC, ESR & HB along with control set.

- Study of histopathalogical alternation due to effect of chlorpyrifos in liver, gill and muscles.

- Study of biochemical alterations on proteins, glycogen, glucose, amino acids and lipids of muscle, liver, kidney & brain under influence of Chlorpyrifos insecticide.

METHODOLOGY TO BE ADOPTED

The experiments would be performed at different exposure times and their effects are evaluated in the following different physiological and biochemical parameters of the fish by using different standard methods & techniques.

(1) Toxicity Evoluation

Healthy fresh water fish *Labio rohita* will be collected from the local fish market and divide fishes into different sets and kept in aquariums. To avoid any dermal infection 1% KMnO4 solution will be added Toxicity Evaluation will be carried out by the fenny method (1971).(17)

(2) Respiratory Metabolism

The respiration metabolism will be studied by Winkler's method & by saroja (1959). (18)

(3) Hematological Alterations

To assess toxic effects on hematological parameters such as (a) RBC, WBC count by using Neuberger hemocytometer. (b) Estimation of Hb will be carried out by Sahli's method. (c) ESR estimation will be carried out by Wintrobe method (1973). (19)

(4) Histological Alternations

Fresh tissues of the selected organs (gill, muscle and liver) will be dissected and preserved.
Histological slides will be prepared of the above organs from experimental as well as control fish tissue.
Digital photography of the histological slides using light microscopy.

(6) Biochemical Alterations

(a) Protein estimation will be carried out by the method of Lowry et al (1951). After the exposure of 24,48,72,96 hrs the protein estimation will be studied.The Protein will be expressed in terms of mg of protein/gm. wet weight of tissue. (21)

(b) Glucose and Glycogen estimation will be carried out by method anthrone method of Caroll et al(1956). (26) After the exposure of 24,48,72,96 hrs the glucose and glycogen estimation will be studied. The values of Glucose and Glycogen content will be expressed in terms of mg of Glycogen / gm. wet weight of tissue. (22)

(c) Amino Acids estimation will be carried out by the Ninhydrin method. After the exposure of 24,48,72,96 hr amino acid estimation will be studied. The values of amino acid will be expressed in terms of mg of amino acid/gm wet weight of tissue. (20)

(d) Lipids will be extracted and estimated from tisues by using the Bligh and Dyer (1959) method. After the exposure of 24,48,72,96 hrs the Lipids will be estimated. (23)

IMPORTANCE OF STUDY

1) International status
Fisheries are valuable resources for millions of people globally. pesticides are being used in agriculture more frequently over the world, particularly for the world's ever-growing population. Many researcher from different country studied excess use of pesticides cause many adverse effect on aquatic ecosystem mainly on fishes and indirectly on human health.

2) National status
India is the second largest fish producing country in the world. Fish is a vital source of protein. In India the work done on the impact of pesticides toxicity in fishes and Fishes serve as important bioindicators for aquatic contamination.

3) Regional status
Few studies were done to evaluate the toxic effect of pesticides on biochemical, and histological, reproductive, hematological and respiratory changes in fishes.

SCOPE OF STUDY

1. By using scientific knowledge of current research, the farmers will be learnt.

2. The awareness among the farmers will be increased regarding the use of natural insecticides rather than the use of serious toxic insecticides.

3. The People will be aware of using the fish population affected by insecticides to avoid health hazards among them.

PROPOSED WORK PLAN/FORMULATION & STRUCTURE OF STUDY

Year Wise Plan Of Research Work

First Year

1. Review of Literature, collection of references from various sources and study of freshwater fish *Labio rohita*.

2. Chemical parameter analysis of water sample (a medium in which the Subject of this investigation inhabits).

3. Study of toxicity evaluation i.e. determination of LC50 value under influence of Chlorpyrifos insecticide compared with control.

Second Yea

1. The study of Histological alternation in liver, gill and muscle of fish after chlorpyrifos insecticide treatment.

Third Year

1. The study of biochemical parameters includes alterations of Proteins, Glucose, Glycogen, Amino Acids and Lipids in different tissues of *Labio rohita*.

2. Analysis of statistical data, documentation and presentation of data, Calculations, results, discussions, conclusion and summary. Preparation of synopsis and DTP work of thesis.

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